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to the

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

on a

Fundamental Investigation of Losses of Skeletal Mineral in Young Adult Human Males and Collaterally in Young Adult Male Pigtail Monkeys (Macacus Nemestrima) Through Immobilization for Varying Periods of Time, Coupled With a Study of Methods of Preventing or Reducing Mineral Loss

(Grant Number NsG-440)

from the

NELDA CHILDERS STARK LABORATORY FOR
HUMAN NUTRITION RESEARCH

TEXAS WOMAN'S UNIVERSITY

September 30, 1963

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SEMIANNUAL REPORT [1 Apr. - 30 Sep. 1963]

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TEXAS WOMAN'S UNIVERSITY

Denton, ~~Texas~~

Pauline Beery Mack et al

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FIRST SEMIANNUAL REPORT

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In the research program at TWU on losses of skeletal mineral in young adult human males and collaterally of young adult pigtail monkeys (Macacus Nemestrima) through immobilization, coupled with a study of methods of reducing mineral loss, the following has been accomplished during the first six months of the study.

After screening approximately 20 men through medical, biochemical, and psychological means, four men were chosen for the early phases of the investigation. For the first eight weeks the men were ambulatory, residing in the Nutrition Clinic and eating on the campus, with a diet provided which was high in protein, calcium, phosphorus, magnesium, iron, and major vitamins.

The men were given duties in the research laboratory which they were required to fulfill at all times when they were ambulatory. They were given week-end leave from Saturday afternoon until Monday morning if they cared to leave the campus, with the understanding that they would try to follow their prescribed diet.

Blood tests were made frequently to ascertain hematological levels, and levels of major nutrients. Radiographs of the os calcis (lateral) were made frequently, with the lumbar spine x-rayed about once weekly.

The ambulatory period of the study served to show that the skeletal system is highly dynamic, with changes in skeletal density evident after only a few hours. Variables related to bone density were found to include physical activity and diet, and probably other factors.

During the period covered by this report, the staff at TWU cooperated in two bed rest studies conducted at the Texas Institute of Rehabilitation and Research at the Houston Medical Center to the extent of conducting measurements of radiographic bone densitometry and analyses of calcium and phosphorus in the urine. Analysis of data of the first study, involving six men in two three-day bed rest periods, nears completion. The second study, which included six men on two 14-day bed rest periods, was finished in September, although analysis of the extensive data collected was far from complete.

The first of the two Houston studies has shown that bed rest even of as short a time as three days was followed in all men by a loss in bone

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mass in the os calcis. After a 10-day interim period, a second three-day bed rest period in which controlled isometric exercise was given produced a bone mass status in the central section of the os calcis which was significantly increased.

In an initial primate study, bone mass in numerous parts of the body as measured from radiographs were significantly higher following a period in which vigorous exercise was taken under direction. Balance studies constituted an accompaniment to the radiographic measurements.

The primate balance studies have shown the value of including feces as well as urine in the measurement of elements excreted, since the latter markedly exceeded the former in quantities of calcium and of phosphorus. In these laboratories feces as well as urine always are included in a balance study, in view of the fact that this situation has been encountered much earlier.

The distinct value of conducting primate studies collaterally with human studies in the field of radiographic bone densitometry has been shown in this part of the investigation. Author

AMBULATORY PHASE OF TEXAS WOMAN'S UNIVERSITY BED REST STUDIES

During the early phases of the Texas Woman's University studies on losses of skeletal mineral in young adult human males resulting from immobilization, it was decided that the mass of certain anatomical sites as measured radiologically should be followed for a period approximately of eight weeks while the subjects were ambulatory and were consuming an optimum diet.

Selection of Subjects

Approximately 20 men were screened by the following means:

(a) Medical examinations including lung x-rays, electrocardiograms, blood and urine tests, and physical examinations at the Medical-Surgical Clinic of Denton, Texas, where our consulting physician and consulting radiologist practice;

(b) Psychological tests and interviews;

(c) Initial radiographs of os calcis, hand, and lumbar spine, and initial blood tests for calcium, phosphorus, and major vitamins.

From these tests four men were chosen for the study, and one man who was rejected as an experimental subject because of medical findings was chosen as a daytime orderly for the bed rest phases of the investigation. A night orderly was selected later.

All of the men who were selected had completed a part of their college or university training and were in need of funds. One man, Subject A, had had seven years of experience on his father's farm and ranch near Sanger, Texas, after high school and had held other jobs and had had some university training.

Subject B had completed the B.S. degree in Education and had taught children in an elementary school near a logging site in Alaska. He expects to go on to graduate school.

Subject D had completed approximately one-half of his undergraduate work in Chemistry at the University of Texas and had worked intermittently while attending the university.

Subject E had worked as a musician and had begun training in statistics.

The man who was selected as an orderly was dropped as an experimental subject because an early childhood mild case of infantile paralysis had left a permanent mark on one arm. On the basis of the results obtained from initial x-rays coupled with advice from physicians, he was not continued as a subject. Because it was believed that an orderly should understand all phases of the research project so that he could carry on his duties with intelligence, this man was continued on the project on this basis. He was a pre-legal university student striving to earn some funds so that he could continue through his legal training. The four men chosen for the study ranged in age from 22 to 28 years.

<u>Duties Assigned Experimental Subjects</u>
<u>while they were Ambulatory</u>

Each of the four experimental subjects and the man designated as an orderly were assigned a job in the laboratories of the Research Foundation during all periods when they were to be ambulatory. The assignments were made on the basis of interviews with the men. Subject A was given a duty in the Textile Technology Laboratories. Because of his family association with cotton growing, he expressed an interest in a cotton project sponsored by the United States Department of Agriculture.

Subjects B and E elected to work in the Statistical Laboratories of the Foundation, which also gave them some insight into the workings of the TWU Data Processing Center.

Subject D was assigned to the Biochemistry Laboratories of the Nelda Childers Stark Laboratory for Human Nutrition Research as a glassware cleanser and general helper.

Assignment of the men to duties in the various TWU Foundation Laboratories made it possible to have them at hand when radiographs and various biochemical tests were to be made. Sleeping in the Nutrition Clinic tended to standardize hours of sleep.

Dietary Plan for the First Phase of the Ambulatory Study

An initial series of blood tests was made for the purpose of finding any possible non-optimum values which would indicate that certain nutrients should be emphasized in the diet. The hematological blood values tended to be high, consistent with the fact that the past diets of all four men, obtained roughly by recall, were high in meat. The lowest values were those for plasma ascorbic acid. Except for one of the four men, these values were marginal to very low.

During the phase of the study covered by this report, the experimental subjects were fed in the student Dining Rooms. They were given a Plan for Food Selection at conferences which were held frequently by the Director and staff members.

The Dietary Plan included the following basic menus for all four subjects:

Breakfast

Cereal (cooked or ready-prepared)

Milk and sugar for cereal

2 eggs

Ham or Canadian bacon

Milk (1/2 pint or more if desired)

6 oz. orange juice fed in laboratory
after return from the Dining Room
(Note: Another 6 oz. glass of orange
juice was fed during the day at the laboratory)

(Note: Coffee or tea may be added, but not as substitutes for milk)

Lunch

A mixed dish containing about 1/2 serving of meat, such as ham and pineapple, turkey or chicken a la king, salmon croquettes, tuna fish salad

One bright yellow or bright green vegetable

One other vegetable

Fruit dessert

Milk (one pint)

(Note: Coffee or tea may be added, but not as substitutes for milk)

Dinner

One meat, fish, or poultry dish (liver once or twice a week)

Small serving of potatoes, rice, or macaroni and cheese

One green or yellow vegetable

Salad - fruit or tossed, with plentiful dressing

Choice of desserts

Milk (1/2 to 1 pint)

(Note: Coffee or tea may be added, but not as substitutes for milk)

Weights of representative servings of food as consumed by the subjects in the Student Dining Room were obtained by staff members, and records of food eaten were kept by the men. A staff member ate with the men for many of the meals in order to advise them.

Thirty meals matching those selected by the men during this period were frozen for analyses of protein, calcium, phosphorus, magnesium, and vitamin A. The ascorbic acid content of the orange juice (each six-ounce portion of which was made up of one part frozen juice to two parts water) was determined analytically periodically during the study.

The laboratory analyses of the foods have not yet been completed. From calculations based on tables of food composition, used temporarily, it seemed evident that the meals consumed by the experimental subjects during this period met their caloric requirements as evidenced also by their body weights. Also, on a calculation basis, the intake of major nutrients surpassed the Recommended Allowances of the Food and Nutrition Board, National Research Council, in all respects. After the laboratory analyses have been completed, this statement may be revised, since analytic values for food composition will be substituted for average values obtained from tables.

Analyses of blood were made initially and periodically throughout the study. The data for the first test and for a test made three weeks after this phase of the study began are given in Table I (pages 9 and 10).

The table shows that the hematological values of the experimental subjects, initially high, remained high after three weeks on their diets.

The three men who initially were very low in plasma ascorbic acid had increased markedly in this blood value when they were tested after three weeks. For example, Subject A rose from 0.3 to 1.6 mg. per 100 ml., Subject D from 0.2 to 0.9, and Subject E from 0.3 to 1.5 mg. per 100 ml. of plasma. Subject B, initially high, remained at the same level.

Plasma vitamin A was relatively high throughout in all subjects. Plasma carotene increased in three of the four subjects. Serum total protein and serum albumin were satisfactory initially and in the three-week test.

Serum calcium and plasma phosphorus were satisfactory at both tests, although calcium increased in two subjects.

In this presumably optimum diet made available to the men during this phase of the program, particular attention has been paid to offering protein which provides all essential amino acids, to iron and magnesium as well as calcium and phosphorus, and to the major vitamins. Particularly ascorbic acid, vitamin A and vitamin D have been shown in these and in other laboratories to be related to skeletal mineralization.

Magnesium is regarded as requiring attention, since a deficiency in this nutrient has been found to affect calcium metabolism adversely.

A study of the effects of the major nutrients which are related to the mechanics of skeletal mineral metabolism will be a part of this research project.

Ambulatory Phase of the Investigation and Bone Density

In order to find the level or levels of bone density in the anatomical sites selected for investigation in each of the experimental subjects while they were ambulatory, they were kept at their assigned laboratory jobs for eight weeks before the bed rest studies were initiated, as noted. Radiographs of the os calcis (lateral view) were made at frequent periods during this time, with radiographs of the lumbar spine (lateral view) made approximately once weekly.

When the prospective subjects were being interviewed in order to select those who would be chosen for the study, it was found that it would be acceptable to the men to house them in the Clinic for entire periods of bed rest and for other times when balance studies were being made. For the long initial ambulatory phase of the investigation, however, it became evident that the selection of subjects would be markedly limited unless week-end leaves were allowed.

On this basis the subjects who were chosen worked at their respective jobs eight hours daily and four hours on Saturday morning. They were excused to go to their homes after lunch on Saturday until eight o'clock the following Monday morning. They could eat intervening meals in the university dining room, if they chose, or they could eat elsewhere if they would attempt to follow their dietary pattern.

Many situations developed over week-ends which yielded valuable data.

TABLE I

BLOOD TESTS RELATED TO NUTRITIONAL STATUS OF EXPERIMENTAL SUBJECTS

Test	Subject A I[1]	Subject A II[2]	Subject B I	Subject B II
Hemoglobin (g/100 ml. blood)	15.8	15.6	15.7	16.0
Red Cell Count (millions/cu. mm. blood)	6.84	6.65	6.62	6.74
Hematocrit (per cent)	43	44	44	44
Ascorbic Acid (mg./100 ml. plasma)	0.3	1.6	1.1	1.0
Vitamin A (mcg./100 ml. plasma)	55.7	62.3	52.3	54.5
Carotene (mg./100 ml. plasma)	0.107	0.200	0.090	0.108
Total Protein (g./100 ml. serum)	7.24	7.39	7.98	7.64
Albumin (g./100 ml. serum)	3.41	3.50	3.12	3.17
Calcium (mg./100 ml. serum)	9.2	10.5	9.5	9.7
Phosphorus (mg./100 ml. plasma	3.8	3.9	4.3	4.1

[1] Initial Test

[2] Test after three weeks on improved diet

TABLE I, CONTINUED

BLOOD TESTS RELATED TO NUTRITIONAL STATUS OF EXPERIMENTAL SUBJECTS, CONTINUED

Test	Subject D I[1]	Subject D II[2]	Subject E I	Subject E II
Hemoglobin (g./100 ml.)	15.8	15.8	15.0	15.5
Red Cell Count (millions/cu. mm. blood)	6.04	6.32	6.04	6.10
Hematocrit (per cent)	43	45	45	45
Ascorbic Acid (mg./100 ml. plasma)	0.2	0.9	0.3	1.5
Vitamin A (mcg./100 ml. plasma)	61.2	55.7	49.3	55.3
Carotene (mg./100 ml. plasma)	0.158	0.137	0.120	0.143
Total Protein (g./100 ml. serum)	6.99	7.19	6.76	6.86
Albumin (g./100 ml. serum)	3.52	3.50	4.05	4.05
Calcium (mg./100 ml. serum)	9.5	9.5	9.1	9.9
Phosphorus (mg./100 ml. plasma)	4.4	4.7	4.9	5.0

[1] Initial Test

[2] Test after three weeks on improved diet

Variations in Os Calcis Density during Ambulatory Periods

Throughout the eight weeks during which the subjects were ambulatory, various situations arose in which the skeletal mass of the central os calcis section was affected. Several of these situations are discussed in the pages which follow.

The change in skeletal mass in the bone being studied radiographically when the diet underwent improvement is shown in Figure 1. Here the change was highly favorable.

The adverse effect of a common cold which beset Subject E for two and one-half weeks, which involved two short periods of bed rest, is shown in Figure 2.

Figure 3 illustrates the loss in skeletal density occasioned by a late birthday party celebrated by Subject A on Friday night, followed by bed rest Saturday morning except for the period required to come for x-rays, with additional bed rest Saturday afternoon and most of Sunday. A departure from the recommended diet also took place Saturday and Sunday.

Figure 4 shows the effect upon skeletal mass in the os calcis of a week-end motor trip by Subjects A and D, even though an attempt was made to follow the prescribed dietary pattern.

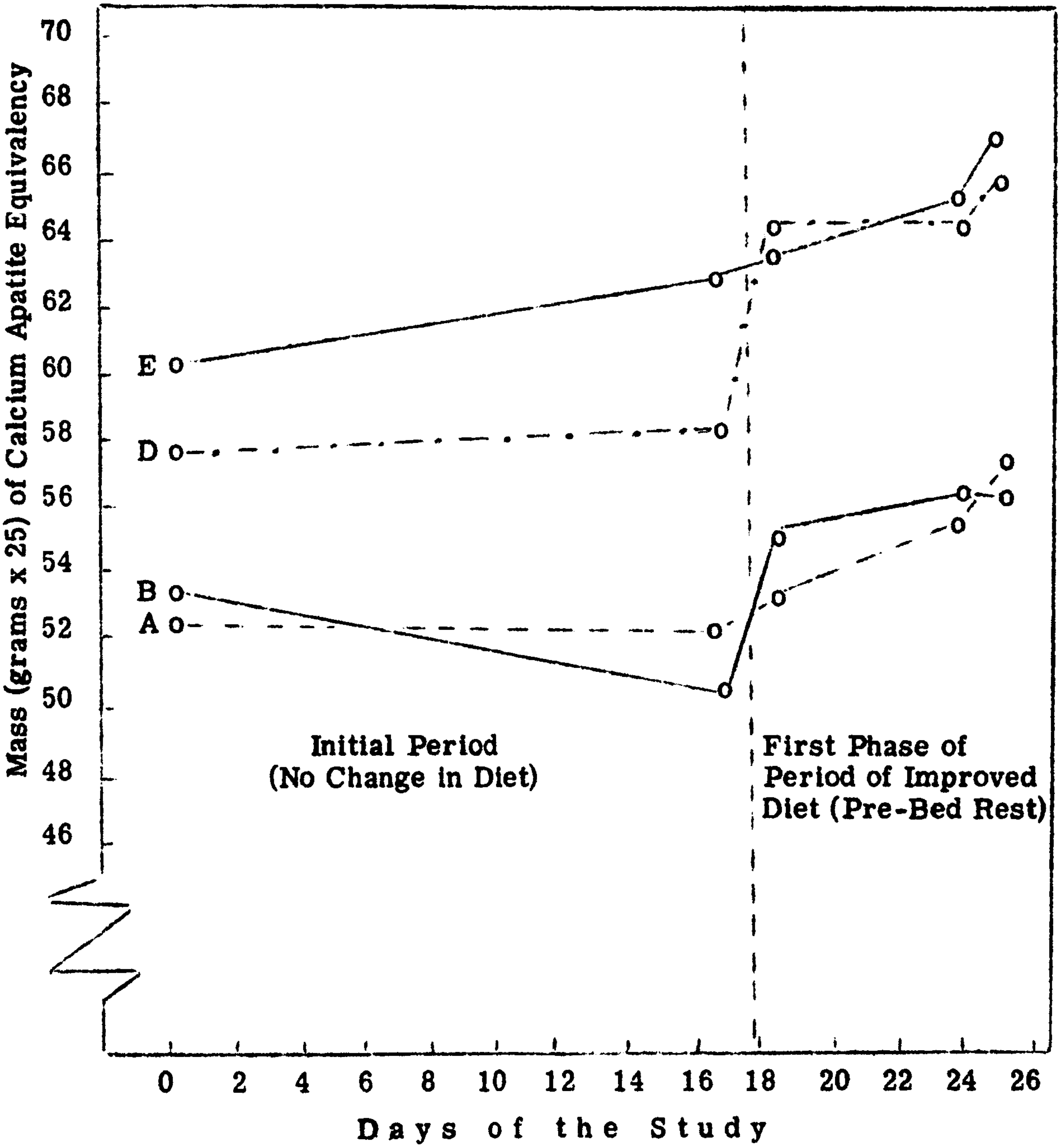


Figure 1

Improvement in Os Calcis Bone Mass was shown by all four subjects when a general improvement in the diet was effectuated, including a marked increase in calcium.

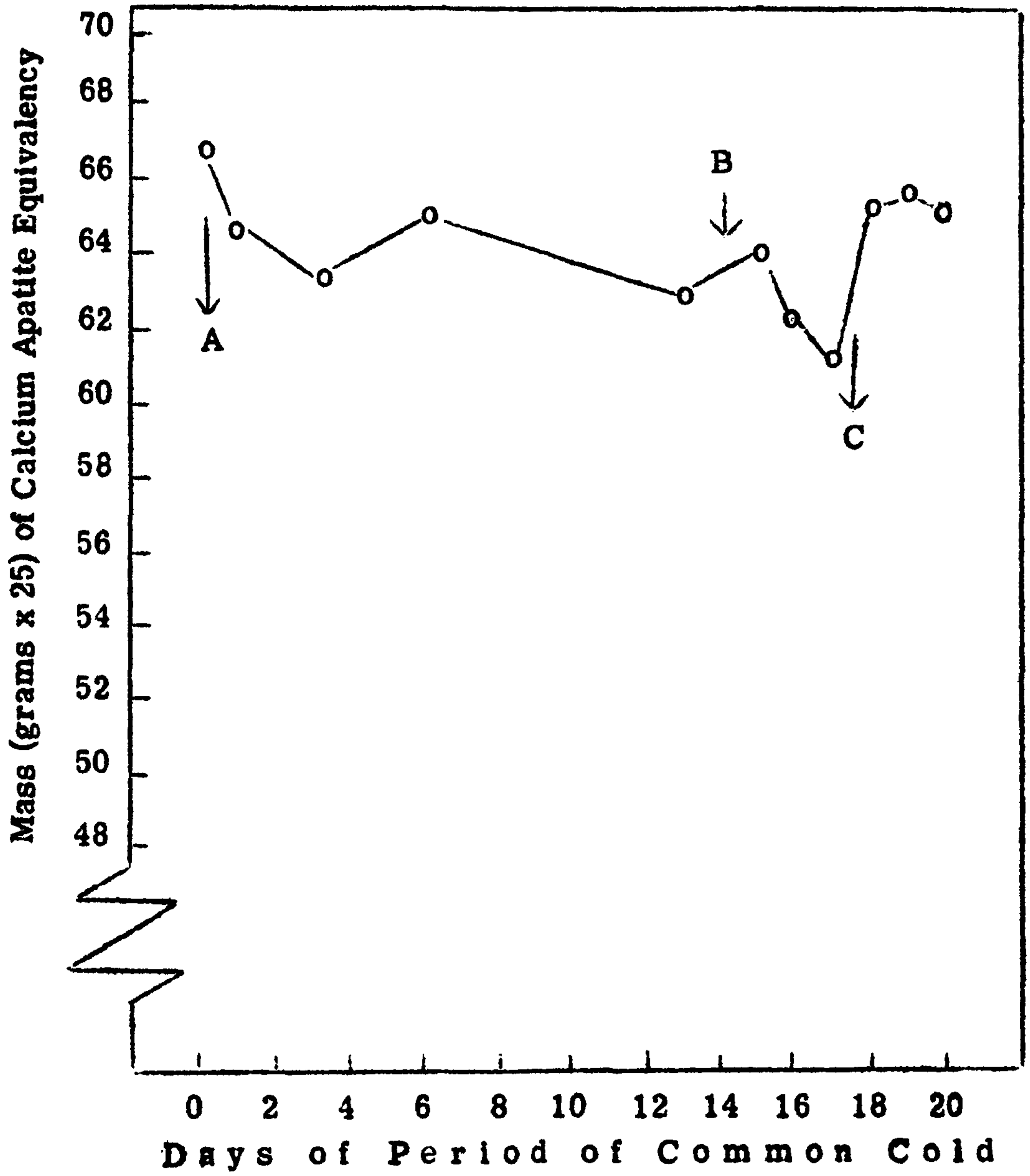


Figure 2

Mass of central os calcis section of one subject (E) during one part of the Pre-Bed Rest Period, when the subject had a common cold through a succession of days. On the first afternoon of this series of days (Point A) the first appearance of the cold became evident. The subject rested in bed for most of the first three days and then was up and around but not active for ten days, with the cold still in evidence. Then at Point B he went to bed for three days. He arose at Point C and resumed regular activities.

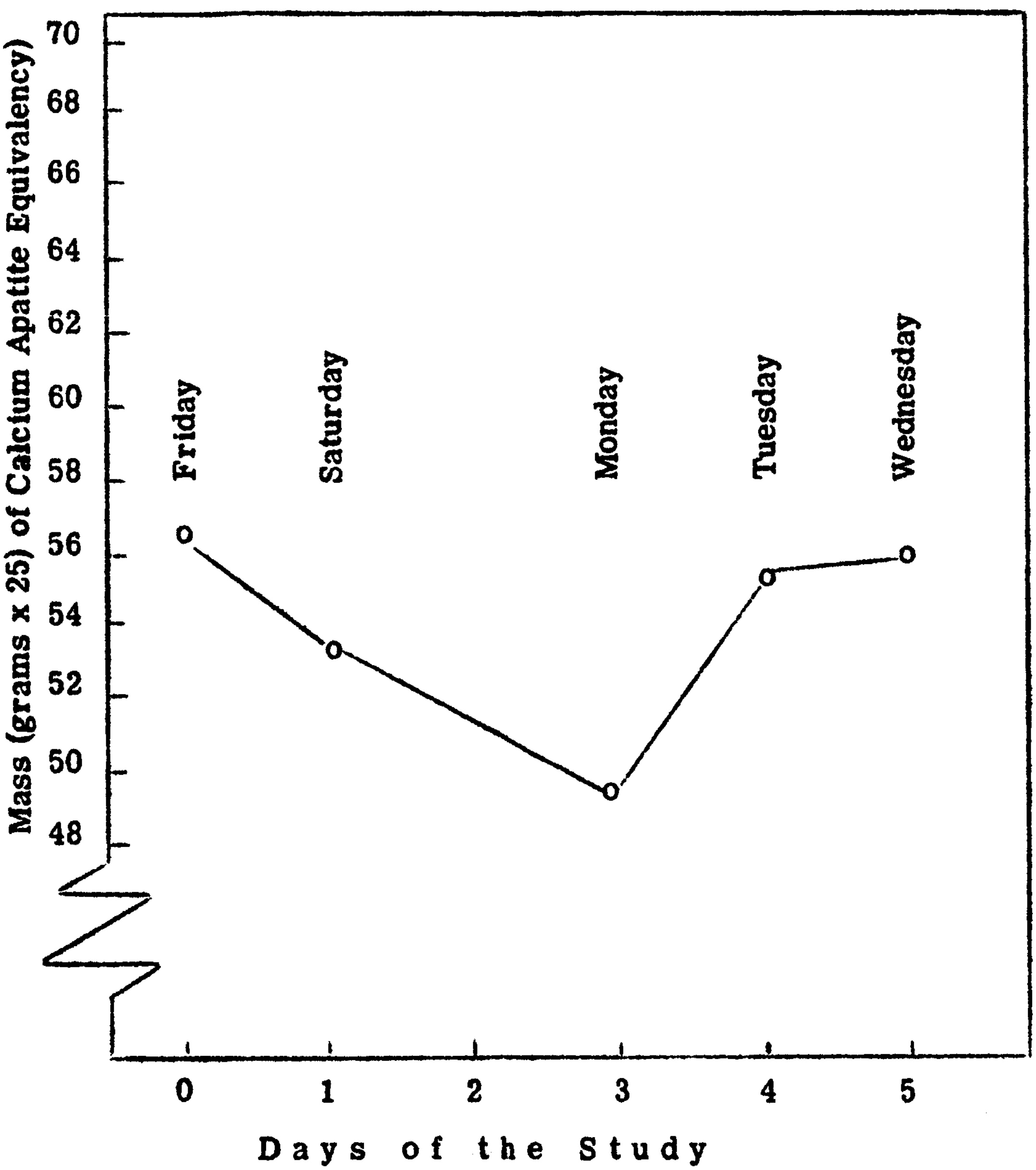


Figure 3

Effect of birthday celebration by Subject A during Pre-Bed Rest Ambulatory Period. The celebration took place Friday night, with voluntary bed rest and lapse in diet conformity over subsequent week end.

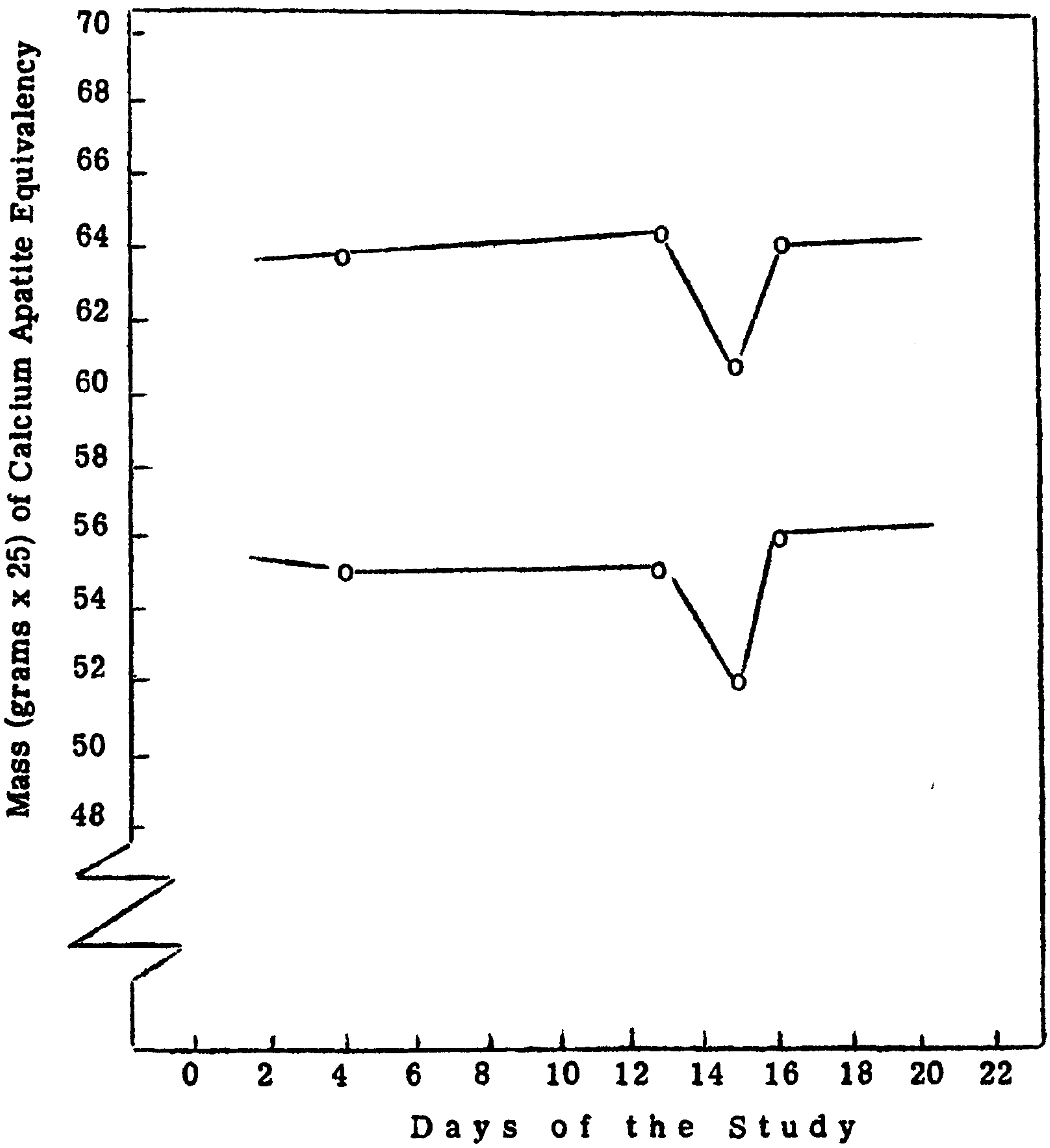


Figure 4

Effect of a week-end automobile trip made by Subjects A and D, involving a total of 22 hours of driving. During the 48 hours of absence from the TWU campus, most of the time was spent sleeping when the subjects were not driving. An attempt was made to maintain the dietary pattern which they were following.

Effect of Improved Diet
on Os Calcis Mass

Density of an object is the mass of the object per unit volume. Since the volume of a bone in an adult subject not suffering from any type of bone disease is not changing, the results in this study are given in terms of mass. Since the standardization wedges used in the radiographic work of this laboratory are calibrated in terms of calcium apatite, $3Ca_3(PO_4)_2 \cdot CaCo_3$, the results are reported as grams of calcium apatite equivalency.

The site which has been measured at the time when this report was due is a central section of the os calcis from a posterior to an anterior landmark. Other sections of this bone parallel to the central section will be evaluated later, but the current report is based on this posterio-anterior section. Also, vertebrae of the lumbar spine are undergoing skeletal mass radiographic measurements, which will be reported on at a later time.

It has been found from numerous studies made in this laboratory that the os calcis, as a weight-bearing part of the anatomy, is readily amenable to changes in skeletal mass from dietary and other environmental changes.

In this study, the improved diet made available to the subjects during the ambulatory phase of the study resulted in an improvement in skeletal mass in the central section of the os calcis which was investigated, as seen in Figure 1.

The initial values in the graphs were those obtained from the four experimental subjects at the time when they were screened. Sixteen days later, when the study began, the initial values had changed very little. After the improved diet was provided, the skeletal mass improved for all subjects. The greater values for Subjects D and E result from their greater dimensions of the os calcis.

The values upon which the graphs in Figure 1 are based were the following:

Mass in Terms of Calcium Apatite Equivalency (grams x 25)

	Subject A	Subject B	Subject D	Subject E
Initial	5.220	5.360	5.791	6.014
After 16 Days . .	5.205	5.042	5.816	6.311
Test after New Diet (1)	5.363	5.527	6.441	6.345
(2)	5.536	5.615	6.427	6.508
(3)	5.611	5.615	6.572	6.669

Although the serum calcium values did not show marked changes during this period, the dietary calcium increased markedly as did the dietary levels of certain of the vitamins, particularly ascorbic acid.

Serum calcium levels tend to show only small changes within a short period of time, even if changes in the levels of dietary calcium are marked. Plasma ascorbic acid, on the other hand, undergoes speedy and marked increases following elevations in dietary intake.

Location of Greatest Changes in Skeletal Mass in the Central Section of the Os Calcis

Because the central posterio-anterior section of the central section of os calcis is composed of an outer relatively thin layer of cortical tissue which envelops an elaborate network of trabecular or cancellous tissue, an effort has been made to find the portions of the section which exhibit the greatest change in skeletal density from one test period to another. For this purpose, the values are taken from the densitometer while the scanning is in progress, at intervals each of which represent one-tenth of the tracing path. This gives a readout count for this segment which can be translated into mass.

Figure 5 shows a positive of the radiograph of the lateral view of an os calcis. Figure 6 shows a diagram of the cross-section which is scanned at the path denoted in Figure 5, divided into 10 segments. The segments are labeled from (a) to (j) to correspond with the values given in certain tables. The diagram in Figure 6 is superimposed over the radiograph of the central section of a cadaver os calcis at the position of the tracing path.

Figure 5

Conventional Trace of Posterio-Anterior Central Os Calcis Section

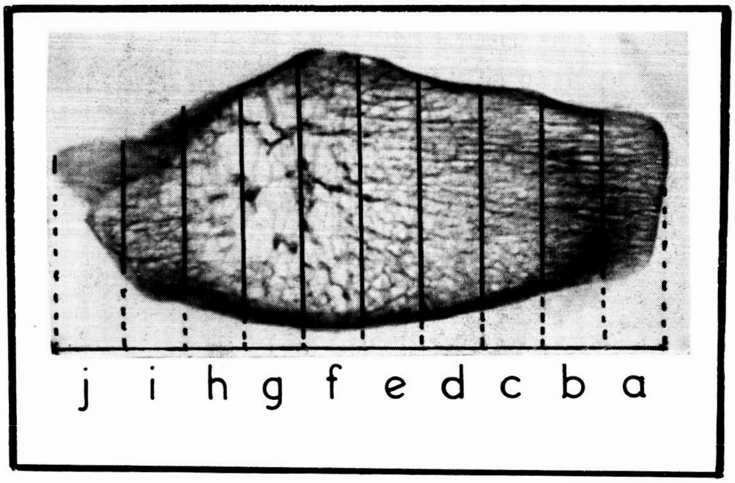
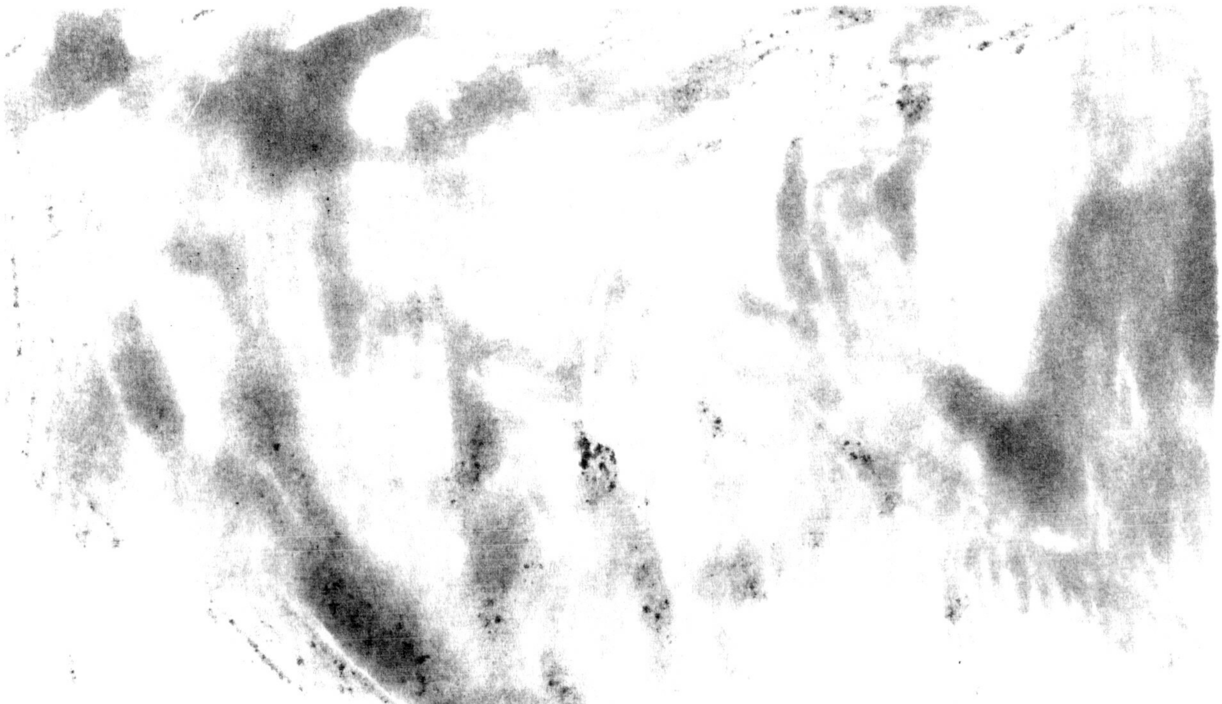


Figure 6

Section of Os Calcis Penetrated by X-Rays and Traced in Densitometer in Path Shown in Figure 5, together with Ten Segments upon which Calculations are Based



The data given on page 17 are based on the sum of the mass values for the entire central os calcis sections. Table II gives the values for the mass of each one of the 10 segments of each os calcis central section for the four respective subjects at the time when the improved diet was introduced in the ambulatory phase of the study, and again after eight days on the diet.

<u>Each of the 10 segments of the central os calcis section of each subject showed an increase in mass after eight days on the improved diet.</u> The diets were improved chiefly in the amount of milk, orange juice, vegetables other than potatoes, and salads which were consumed. Lean meat, fish, and poultry, initially high in the menus of all of the four men, remained high.

Distinct individualism was shown by the several men in the extent to which they exhibited an increase in skeletal mass of the os calcis section under discussion. Subjects B and D experienced the greatest overall percentage change, 11.36 and 13.00 per cent, respectively, for the increase in mass for the section as a whole. Subject A increased 7.80 per cent and Subject E 5.67 per cent in bone mass for this section.

This finding is consistent with the representative diets which the men reported as pertaining before they came on the study.

There were no consistencies for the four men as to which of the 10 segments within the central os calcis section showed the greatest change.

TABLE II

PER CENT CHANGE IN SKELETAL MASS OF TEN SEGMENTS OF THE CENTRAL OS CALCIS SECTION DURING THE INITIAL EIGHT DAYS OF DIET IMPROVEMENT

(Mass in grams calcium apatite equivalency x 25)

Segment of Os Calcis	Last Test before Improved Diets (1)	Test Eight Days after beginning Improved Diets (2)	Per Cent Change from Test (1) to Test (2)
SUBJECT A			
(a)	0.249	0.271	+ 8.83
(b)	0.357	0.375	+ 5.04
(c)	0.459	0.500	+ 8.93
(d)	0.552	0.593	+ 7.43
(e)	0.606	0.664	+ 9.57
(f)	0.628	0.694	+10.51
(g)	0.636	0.651	+ 2.36
(h)	0.588	0.626	+ 5.46
(i)	0.584	0.629	+ 7.65
(j)	0.546	0.608	+11.36
Entire Section	5.205	5.611	+ 7.80

TABLE II, CONTINUED

PER CENT CHANGE IN SKELETAL MASS OF TEN SEGMENTS OF THE CENTRAL OS CALCIS SECTION DURING THE INITIAL EIGHT DAYS OF DIET IMPROVEMENT

(Mass in grams calcium apatite equivalency x 25)

Segment of Os Calcis	Last Test before Improved Diets (1)	Test Eight Days after beginning Improved Diets (2)	Per Cent Change from Test (1) to Test (2)
SUBJECT B			
(a)	0.299	0.345	+15.38
(b)	0.399	0.450	+12.78
(c)	0.492	0.548	+11.38
(d)	0.546	0.615	+12.64
(e)	0.548	0.625	+14.05
(f)	0.554	0.614	+12.63
(g)	0.549	0.618	+12.57
(h)	0.560	0.617	+10.18
(i)	0.553	0.593	+ 7.23
(j)	0.542	0.590	+ 8.86
Entire Section	5.042	5.615	+11.36

TABLE II, CONTINUED

PER CENT CHANGE IN SKELETAL MASS OF TEN SEGMENTS OF THE CENTRAL OS CALCIS SECTION DURING THE INITIAL EIGHT DAYS OF DIET IMPROVEMENT

(Mass in grams calcium apatite equivalency x 25)

Segment of Os Calcis	Last Test before Improved Diets (1)	Test Eight Days after beginning Improved Diets (2)	Per Cent Change from Test (1) to Test (2)
SUBJECT D			
(a)	0.348	0.398	+14.37
(b)	0.433	0.480	+10.85
(c)	0.514	0.563	+ 9.53
(d)	0.580	0.644	+11.03
(e)	0.719	0.727	+ 1.11
(f)	0.681	0.790	+16.01
(g)	0.666	0.746	+12.01
(h)	0.650	0.767	+18.00
(i)	0.628	0.738	+17.52
(j)	0.597	0.719	+20.44
Entire Section	5.816	6.572	+13.00

TABLE II, CONTINUED

PER CENT CHANGE IN SKELETAL MASS OF TEN SEGMENTS OF THE CENTRAL OS CALCIS SECTION DURING THE INITIAL EIGHT DAYS OF DIET IMPROVEMENT

(Mass in grams calcium apatite equivalency x 25)

Segment of Os Calcis	Last Test before Improved Diets (1)	Test Eight Days after beginning Improved Diets (2)	Per Cent Change from Test (1) to Test (2)
SUBJECT E			
(a)	0.315	0.320	+ 1.59
(b)	0.517	0.529	+ 2.32
(c)	0.526	0.572	+ 8.75
(d)	0.672	0.692	+ 2.98
(e)	0.746	0.762	+ 2.14
(f)	0.749	0.798	+ 6.54
(g)	0.738	0.755	+ 2.30
(h)	0.713	0.743	+ 4.21
(i)	0.702	0.747	+ 6.41
(j)	0.633	0.751	+18.64
Entire Sections	6.311	6.669	+ 5.67

Effect of a Common Cold and Two Short Periods of Bed Rest on Os Calcis Mass

Figure 2 shows the mass of the central os calcis section of Subject E through 17 days during which he experienced two three-day bed rest periods with an intermediate period of reduced activity. This subject's white cell count through this time was 10,000 to 11,000. The subject attempted to eat the food in his program during this time, with his milk recommendation followed, as well as his intake of orange juice, but with incomplete consumption of other food portions.

The reduction in skeletal mass following both short bed rest periods is evident, as well as the increase in mass when full activity was resumed.

The values for mass of the central os calcis section at various periods covered in Figure 2 were the following:

Mass in Terms of Calcium Apatite Equivalency (grams x 25)

Day before first appearance of the common cold	6.669
After first day of bed rest with the cold	6.458
After two more days of bed rest	6.369
Two intermediate tests while subject was ambulatory, but still suffering from the common cold	6.498 6.349
First day of second bed rest as result of the cold	6.414
After second bed rest day	6.266
After third bed rest day	6.132
Three succeeding ambulatory days with cold apparently gone	6.566 6.571 6.561

Effect of a Birthday Party and Subsequent Bed Rest and Dietary Lapse on Os Calcis Mass

Figure 3 shows the loss of mass in the central section of the os calcis of Subject A following a birthday party on Friday night and a subsequent self-imposed week-end bed-rest and dietary lapse.

Mass in Terms of Calcium Apatite Equivalency

(grams x 25)

Overall mass of central os calcis section Friday A. M.	5.643
Mass of os calcis section Saturday A. M.	5.358
Monday morning after two days of bed rest and neglect of diet .	4.985
Tuesday morning after one day of resumption of regular activity	5.459
Wednesday morning	5.584

The per cent changes in mass of the respective 10 segments of the os calcis section are shown in Table III for the period from Friday morning until Monday morning. The overall reduction in bone mass of the entire os calcis section was 11.66 per cent. The 10 segments of this section experienced losses ranging from 10.21 to 13.76 per cent. The differences in skeletal density in the respective 10 segments were relatively similar throughout.

It is not known how much of the loss in skeletal mass of this section is attributable to the bed rest, or to the failure to maintain the dietary level intended to be followed during this ambulatory period.

TABLE III

PER CENT CHANGE IN SKELETAL MASS OF TEN SEGMENTS OF THE CENTRAL OS CALCIS SECTION FROM FRIDAY MORNING UNTIL MONDAY MORNING WITH AN INTERVENING PERIOD OF BED REST

(Mass in grams calcium apatite equivalency x 25)

Segment of Os Calcis	Test Friday Morning (1)	Test Monday Morning (2)	Per Cent Change from Test (1) to Test (2)
SUBJECT A			
(a)	0.266	0.236	-11.27
(b)	0.376	0.333	-11.43
(c)	0.506	0.451	-10.87
(d)	0.597	0.528	-11.56
(e)	0.676	0.583	-13.76
(f)	0.700	0.614	-12.29
(g)	0.658	0.581	-11.70
(h)	0.634	0.566	-10.73
(i)	0.627	0.563	-10.21
(j)	0.603	0.530	-12.11
Entire Section	5.643	4.985	-11.66

Effect of a Week-End Automobile Trip on Os Calcis Mass

Figure 4 shows the loss in mass of the central os calcis section in two of the subjects during 48 hours between a radiograph made on Saturday and another made on the following Monday, with an automobile trip made to another state intervening. In the 48 hours which elapsed between the two tests, 28 hours were consumed in actual driving, during which time the men alternated in driving and sleeping. During another 14 hours both men slept. An effort was made to eat the desired foods during this week end, but this was not entirely successful as judged from the records which they kept.

The overall loss in radiographically determined bone mass in the center section of the os calcis was not extensive, although a reduction in mass was found in each of the 10 segments of the section of each man. In the case of Subject A, these reductions ranged from 1.70 to 8.96 per cent. For Subject D, the 10 losses ranged from 0.98 to 18.98 per cent.

EQUILIBRIUM IN SKELETAL MASS

The results of the ambulatory tests have shown that an equilibrium in skeletal mass of a certain site in the anatomy can be found for a definite set of environmental conditions, including such factors as diet, level of activity, and possibly the general physical condition of the subject. In the part of the study completed to date, however, it appears that equilibrium is established at one level under a certain dietary program, but that the level differs when the dietary regimen for certain major nutrients has changed.

The deviations in skeletal mass of the os calcis when short time departures from a certain dietary-activity regimen were imposed while the subjects of the study were ambulatory have been instructional. These phases of the study, some of which have been discussed in the previous section of the report, have demonstrated the highly dynamic character of the calcium metabolism process as related to bone.

In the conditions which have caused the rapid changes in the skeletal mass of the os calcis which have been cited, more than one variable has been operative in most cases.

<u>These findings in the ambulatory phases of the study, many of them unexpected, will be followed by controlled tests in which all variables except one will be held constant insofar as possible.</u> For example, it is planned that an automobile drive of a certain time duration will be undertaken with the desired diet packed in sections to be consumed at specified periods of time. A simulation of the birthday party week-end is planned with an attendant administering food periodically while the subject, after preliminary lack of sleep to the point of near exhaustion, is awakened only for short periods to receive his food. The effects of eating the same total amount of food in one or two large installments as compared with this amount in smaller more frequent installments will be studied.

COOPERATION IN FIRST HOUSTON BED REST STUDY

The Texas Woman's University has cooperated during the period covered by this report in two studies conducted by the National Aeronautics and Space Administration at the Institute for Rehabilitation and Research located in the Houston Medical Center, Houston, Texas. The TWU cooperation has consisted in conducting the radiographic work in both studies, and in making urinary analyses for calcium, phosphorus, and nitrogen in the second. study.

The first of the two studies. involved two three-day bed rest periods with an intervening period of 10 days. In the second of the two bed rest periods, controlled isometric exercise was administered. In the second study, the bed rest periods were extended to 14 days each.

The radiographic analyses in the first of these two studies, in which the data began to be collected from 3 May, 1963, near completion with respect to the os calcis. A preliminary analysis of the skeletal density in the lumbar spine has been made, with this to be followed by more detailed densitometric measurements. The densitometric analyses of the x-rays in the second study are in progress, as are the urinary analyses. Analyses of radiographs and urine in the second study are in progress.

As in the case of the studies in progress on the campus of the Texas Woman's University, the skeletal mass in the os calcis has been given distinct attention because this bone is subjected to stress in walking and weight bearing, with this stress markedly reduced with bed rest.

Skeletal Density in the Os Calcis during
Bed Rest, with and without Isometric Exercise

In the first of the two Houston bed rest studies in which we are cooperating, the analyses of the bone mass in the central section of the os calcis have been completed.

Figure 7 shows the bone mass in the four central segments of this section at the beginning and close of each of the two three-day bed rest periods. A similar graph is possible if the entire central os calcis section is included.

The following findings are derived from a study of the figure.

(a) All six men in this study lost mass in the os calcis from the beginning to the close of the three-day bed rest phase.

(b) The men differed in their bone mass in the os calcis during a 10-day interim period. It is assumed that this difference resulted from their respective activities during this period, but only the men's own activity reports are available for this time. Subjects II and VI experienced the greatest gains in skeletal mass in the anatomical site under consideration between the two bed rest periods.

(c) All six subjects were higher in skeletal mass of this bone section at the close of the second bed rest period, during which standardized isometric exercise was administered, than they were when the first bed rest period ended.

(d) Two subjects (V and VI) were lower in skeletal mass of the os calcis at the close of the second bed rest period (with exercise) than at the close of the interim period, although they were higher at the end of this period than at the close of the first bed rest (without exercise), as noted under (c). It is assumed that the exercise involving the os calcis during the interim period was more vigorous than that during the second bed rest.

The percentage difference in skeletal mass of the overall central os calcis section, as well as those of the four designated segments of this section (Segments D, E, F, and G) are shown in Table IV for comparisons of the first and last tests of the first bed rest period and for the last test of the first and the last test of the second bed rest phase of the study.

The table shows that, during the first bed rest period, the six subjects lost skeletal density in the overall central os calcis section in amounts ranging from 8.85 to 22.72 per cent. This overall section had values at the end of the second bed rest (with exercise) ranging from 4.28 to 41.71 per cent higher than those at the close of the first bed rest.

In the four central segments of the center section of the os calcis, losses in skeletal mass ranging from 9.16 to 20.04 per cent occurred for all six men during the first bed rest phase. In the second bed rest with exercise, the final x-ray showed skeletal values which were from 3.36 to 31.82 per cent higher than were the final values during the first bed rest when no exercise was taken.

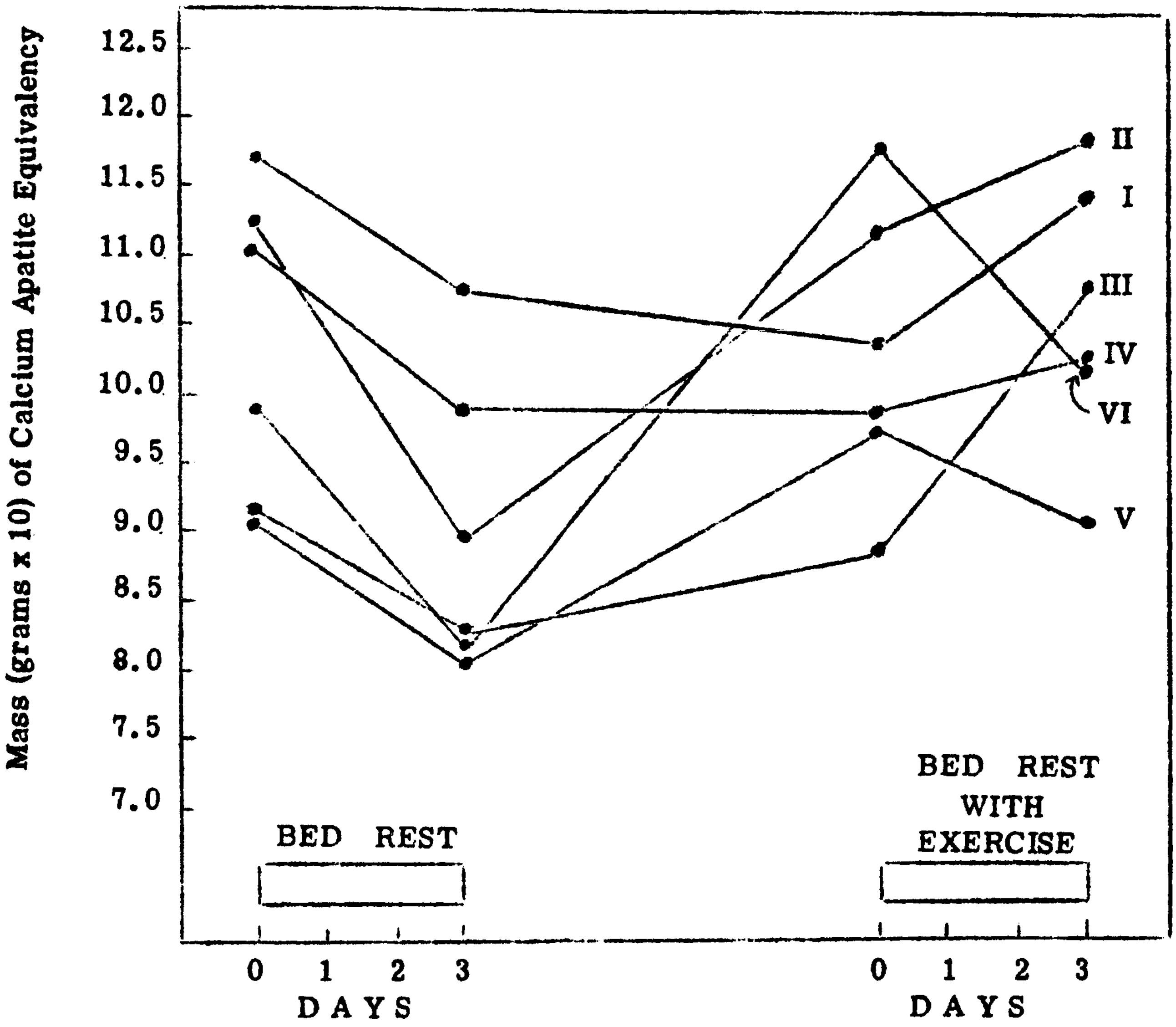


Figure 7

Skeletal mass of the sum of Segments D, E, F, and G of the posterior-anterior central section of the os calcis of six men participating in two three-day Bed Rest Periods separated by ten intervening days.

TABLE IV

PER CENT DIFFERENCE IN SKELETAL MASS OF THE CENTRAL OS CALCIS SECTION AND OF THE MASS OF FOUR COMBINED SEGMENTS OF THIS SECTION BETWEEN DESIGNATED PERIODS OF THE STUDY

Subject	Change from Test 1 to Test 2 during First Bed Rest Period*		Difference between Last Tests of First and Second Bed Rest Periods**	
	Overall Os Calcis Section	Sum of Segments D, E, F, G	Overall Os Calcis Section	Sum of Segments D, E, F, G
I	-11.15%	- 9.16%	+10.14	+ 7.12
II	-22.72	-20.04	+41.71	+31.82
III	- 8.85	- 9.16	+32.58	+29.48
IV	-10.60	-10.93	+ 4.28	+ 3.36
V	-10.66	-10.65	+11.81	+11.81
VI	-18.28	-17.29	+23.38	+24.30

* Extent to which the final test fell below the initial test of the first bed rest period.

** Extent to which the final test of the second bed rest period (with exercise) surpassed the final test of the first period (without exercise).

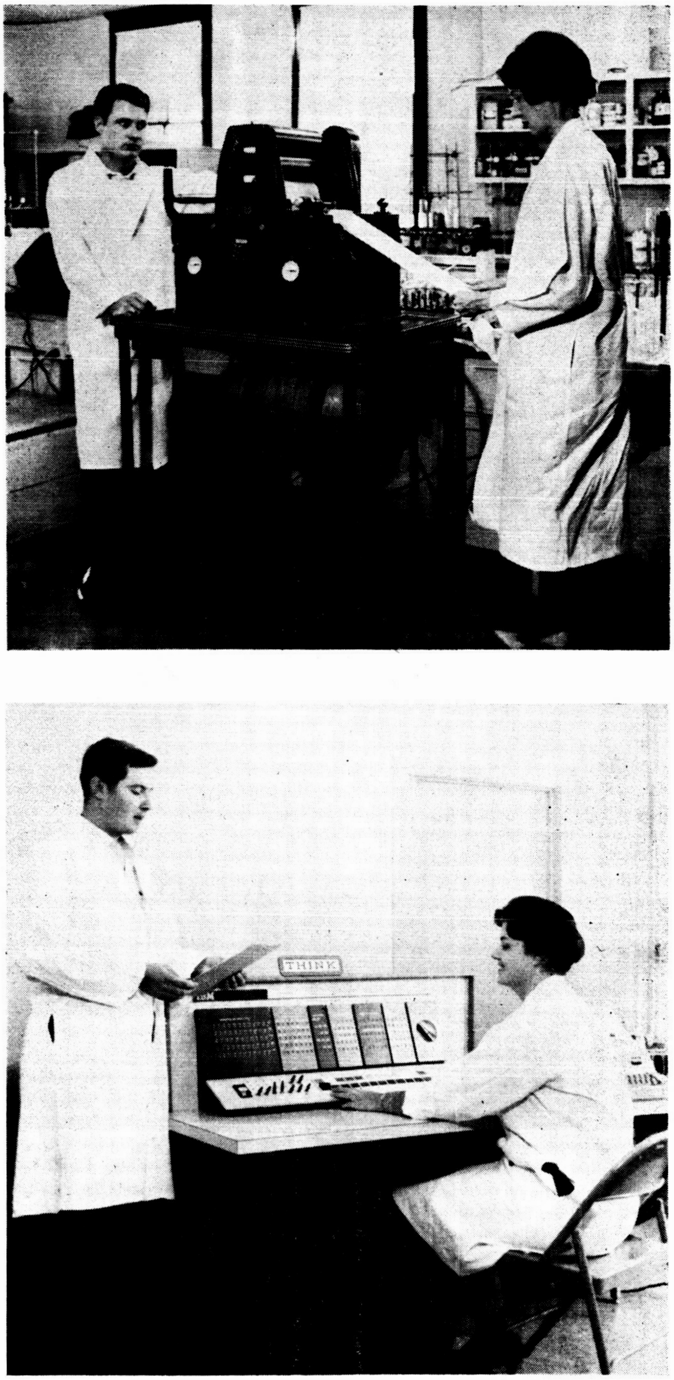


Figure 8

(Above) Subject A assisting Carol Pannell, Textile Technologist, in a Study related to the waterproofing of cotton fabrics.

(Below) Subject B assisting Jessie Ashby, statistician, in the analysis of certain data.

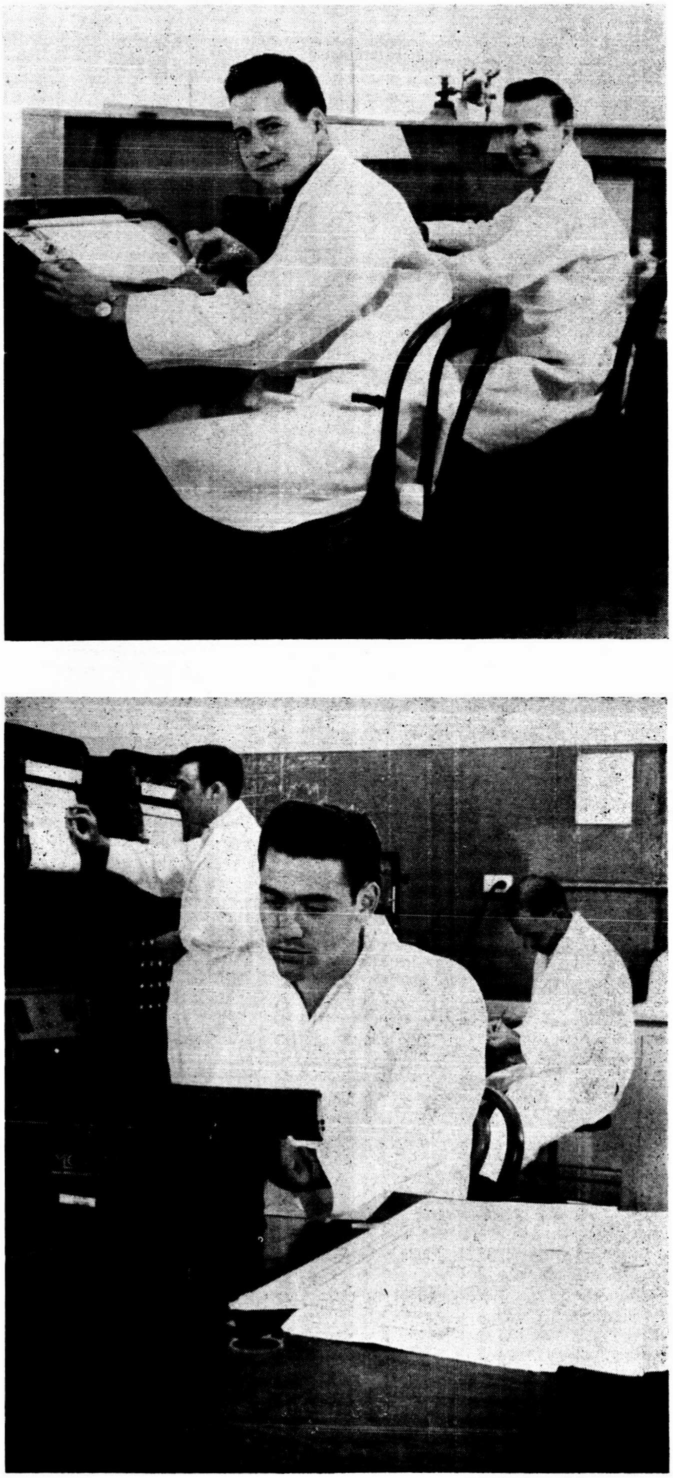


Figure 9

(Above)Subject D assisting Dr.Ralph Pyke, biochemist, in an analytic procedure.

(Below) Subject E operating a calculator in the Densitometer Laboratory, where Mr. Grady Dozier and Mr. Bill Stover are measuring skeletal mass from radiographs by radiographic densitometry.

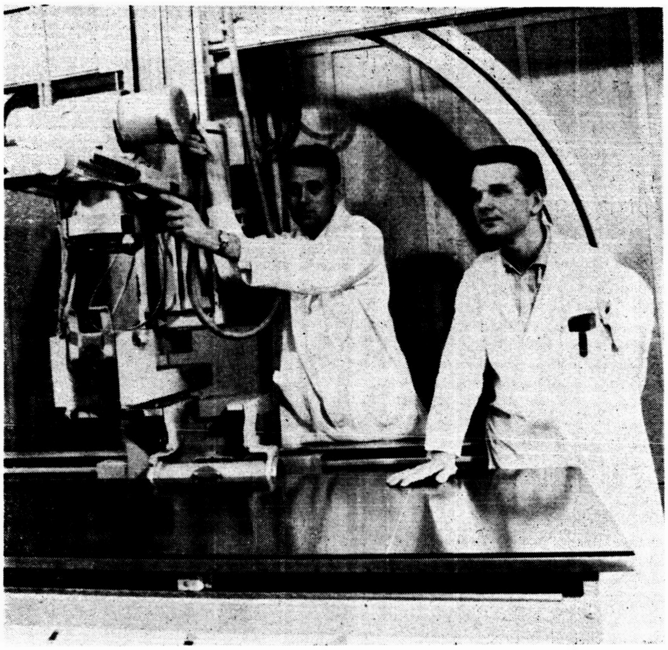


Figure 10

Raymond Knepp (right), who was selected as one of the orderlies for the study, learning the intricacies of the X-ray machine from Professor George Vose ,(left). Mr. Knepp was introduced to all laboratory techniques as an educational procedure.

INITIAL PRIMATE STUDIES

One pigtail monkey (Macacus Nemestrima) served to initiate our Laboratories into radiographic bone densitometric and balance studies with primates, during the period covered by this report. At periods separated by a minimum of one month, five sets of exposures involving six films each were made. These included the following aspects:

(a) Lateral spine

(b) Skull (anterio-posterior)

(c) Left os calcis (lateral)

(d) Left leg (anterio-posterior)

(e) Left knee (lateral)

(f) Hand (anterio-posterior)

For each set of exposures, the animal was given Nembutal in a dosage adequate to give 20 minutes of anaesthesia. The films are undergoing densitometric measurements. Two sets of balance studies involving calcium and phosphorus were run with this primate. In the first study the animal was quietly confined in a metabolism cage throughout. In the second study he was given organized strenuous exercise in his cage twice daily. Food consumption and calcium-phosphorus excretion increased markedly during the second balance trials, although the animal was in positive balance both times.

For both sets of determinations, fecal calcium and fecal phosphorus excretion both were greater than urinary excretion of the same nutrients, indicating the desirability of not estimating losses of these substances from urine alone. The same trend has been found in these laboratories for human subjects.

TABLE V

BALANCE STUDY WITH ANIMAL NOT UNDERGOING EXERCISE

Food Consumption per Week	
Food Weight	grams
Monkey chow	250.0
Apples	1,034.0
Total	1,284.0
Calcium Consumption (by Analysis)	milligrams
Monkey chow	1,935.0
Apples	57.2
Total	1,992.2
Phosphorus Consumption (by Analysis)	
Monkey chow	1,068.6
Apples	116.8
Total	1,185.4
Calcium and Phosphorus Excretion per Week	
Calcium:	
Urine	169.3
Feces	995.3
Total	1,164.6

Phosphorus:

Urine	16.0
Feces	729.5
Total	745.5

Calcium Balance Calculation

1,992.2 - 1,164.6 = + 827.6 milligrams

Phosphorus Balance Calculation

1,185.4 - 745.5 = + 439.9 milligrams

TABLE VI

BALANCE STUDY WITH ANIMAL UNDERGOING STRENUOUS ORGANIZED EXERCISE

Food Consumption per Week

Food Weight	grams
Monkey chow	456.5
Apples	1,163.0
Total	1,619
Calcium Consumption (by Analysis)	**milligrams**
Monkey chow	4,108.5
Apples	64.3
Total	4,172.8
Phosphorus Consumption (by Analysis)	
Monkey chow	2,268.8
Apples	131.4
Total	2,400.2

Calcium and Phosphorus Excretion per Week

	milligrams
Calcium:	
Urine	457.1
Feces	2,340.2
Total	2,797.3
Phosphorus:	
Urine	26.8
Feces	1,591.4
Total	1,618.2

Calcium Balance Calculation

4,172 - 2,797.3 = + 1,375.5 milligrams

Phosphorus Balance Calculation

2,400 - 1,618.2 = + 782.0 milligrams

* * * * * * * *

In common with the higher food intake and excretion values, the bone densitometry evaluations showed higher levels following the period of exercise of the primate than did those following the time of relative inactivity. These limited primate studies demonstrate the undoubled value of primates as a means of securing data from controlled experiments which would supplement human studies in the field of the relationship between skeletal density and various factors such as physical activity. On this basis, the two types of research, on human beings and primates, will continue side by side in the TWU Laboratories.